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EXPLORING GENETIC VARIABILITY IN TOMATO GENOTYPES (*SOLANUM LYCOPERSICUM* L.) USING AGRO-MORPHOLOGICAL AND POLYMORPHISM ANALYSIS OF LYCOPENE BIOSYNTHETIC GENES

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ABSTRACT:
The agro-morphological assessment showed that different generations created different genetic characteristics which affected essential yield and quality measurements. The study found that phenotypic traits showed high similarity to genotypic traits because environmental factors had minimal impact on their development. The study found that yield and fruit weight and locule number traits showed high variability together with their strong inheritance patterns which made them suitable for selection during early breeding generations. The study found that direct phenotypic selection achieved high success because major yield traits showed both high heritability and substantial genetic progress which divided gene effects between additive and non-additive pathways. The study found that all qualitative traits except fruit colour maintained their stability across multiple generations while one mutant line showed a marked change in fruit colour which resulted from specific mutations that affected pigment production. The study confirmed that key genes in the carotenoid pathway undergo EMS-induced mutations which include changes to the lycopene β-cyclase gene. The research showed that nucleotide substitutions resulted in protein alterations which affected essential sections of the protein thus diminishing enzyme function and causing increased lycopene production. Phylogenetic analysis confirmed that treated lines and control lines had separate lineages because both groups had mutations which could be passed down through generations. The research proved that EMS mutagenesis created useful genetic variations which scientists used to conduct genetic and molecular studies. The identified mutant lines serve as valuable genetic materials for breeding programs which aim to develop tomato cultivars with high yields and elevated lycopene content.

KEYWORDS: Genetic advance; genetic variability; genotypic coefficient of variation (GCV); heritability; phenotypic coefficient of variation (PCV); Tomato.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) ranks foremost among vegetable crops in global production, adapting to diverse agro-climatic regimes spanning tropical to temperate zones. As a member of the Solanaceae family, this annual herbaceous species exhibits a diploid chromosome complement of 2n = 24 and predominantly self-pollinating reproductive biology, which underpins its cultivation as a model for autogamous breeding systems. In terms of output, tomatoes trail only potatoes in worldwide vegetable yields (1). From a nutritional standpoint, fruits are replete with provitamin A carotenoids, ascorbic acid (vitamin C), reduced glutathione, and macrominerals including Ca, P, and Fe (2). Notably, tomatoes constitute the principal dietary source of lycopene—a tetraterpenoid carotenoid—alongside ancillary antioxidants; post-version accumulation elevates lycopene concentrations by up to 500-fold, conferring potent reactive oxygen species (ROS)-scavenging efficacy in both raw and processed forms (17). Meta-analyses of cohort studies implicate regular tomato intake in attenuating risks of oxidative stress-mediated pathologies, including prostate carcinogenesis and atherogenic cardiovascular events.

Morphologically, *S. lycopersicum* manifests as an indeterminate, vining perennial in native habitats but is bred for determinate, annual bush forms in agronomy. Erect to decumbent stems (0.6–1.8 m) bear simple to imparipinnate leaves with 3–7 ovate-lanceolate leaflets, often glandular-pubescent for herbivore deterrence. Inflorescences comprise 4–12 perfect, protogynous flowers in cymose racemes, facilitating self-pollination. The indehiscent berry fruit—a multi-locular syncarp—varies in locule number, pedicel articulation, and pericarp thickness across genotypes; mature hues derive from chromoplast accumulation, predominantly all-trans-lycopene yielding scarlet pigmentation.

The autogamous breeding system of tomato curtails intrapopulation heterozygosity, yielding <5% allelic divergence between domesticated accessions and wild relatives, thereby constraining the genotypic reservoir for selection (30). Nonetheless, quantitative genetic variation in agro-morphological descriptors—encompassing plant vigor, inflorescence density, fruit set efficiency, and harvest index—serves as the foundational substrate for phenotypic selection in breeding pipelines. Amplified polymorphism spectra potentiate transgressive segregation and heterotic gains, as corroborated in analogous autogamous systems; for instance, simple sequence repeat (SSR)-based profiling of Sorghum bicolor landraces unveiled high polymorphism information content, delineating clusters for adaptive traits. Phenotypic correlations among sink-source traits (e.g., positive linkage between locule number and total soluble solids) inform index-based selection, optimizing multi-objective gains in yield potential (30), (34), (27). Molecular characterization of key carotenoid biosynthetic genes, including phytoene synthase (PSY1), phytoene desaturase (PDS), ζ-carotene desaturase (ZDS), and lycopene β-cyclase (LCY-B), further strengthens the identification of superior genotypes for enhanced lycopene accumulation and fruit quality improvement.

Limited studies have combined EMS-induced mutagenesis with both agro-morphological evaluation and molecular analysis of lycopene biosynthetic genes in tomato. This study addresses this gap by assessing genetic variability, heritability, and genetic advance in EMS-derived mutant populations of Phule Kesari and Phule Jayshree. Molecular characterization confirmed mutations in the lycopene β-cyclase (CrtL-b) gene associated with changes in fruit colour and lycopene accumulation. The study identifies stable mutant lines with improved fruit quality traits and useful genetic variability, which can be utilized in future tomato breeding programs.

The present investigation was undertaken with the dual objective of (i) estimating genetic parameters (variability, heritability, and genetic advance) for agro-morphological traits in EMS-induced mutant populations of two elite varieties, and (ii) characterizing molecular variation in key lycopene biosynthetic genes to link phenotypic changes with underlying genetic alterations.

MATERIALS AND METHODS

Experimental Site, Plant Material, And Design

Field experiments were conducted at the Instructional Farm of Mahatma Gandhi Mission College of Agricultural Biotechnology (MGMCABT) and Mahatma Gandhi Mission Krishi Vigyan Kendra (MGMKVK), Chhatrapati Sambhajinagar, Maharashtra, India, during the rabi seasons of 2022–23 and 2023–24. The experimental material consisted of two tomato (*Solanum lycopersicum* L.) genotypes, Phule Kesari and Phule Jayshree, developed by the Tomato Improvement Project of Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra, India. Phule Kesari is a determinate cultivar producing medium round orange fruits rich in β-carotene, whereas Phule Jayshree is an indeterminate cherry tomato genotype with round orange-red fruits and higher total soluble solids. Two tomato genotypes (*Solanum lycopersicum* L.), Phule Kesari and Phule Jayshree, were studied to evaluate genetic variability induced through ethyl methane sulfonate (EMS) mutagenesis, along with their respective non-treated controls. The experiment was laid out in a Randomized Complete Block Design (RBD) with three replications (Figure 3 (a) & (b). Although only two genotypes were evaluated, each genotype comprised 800 individual plants, resulting in a total population of 1600 plants. The large within-genotype sample size ensured reliable estimation of phenotypic variability and derived genetic parameters, thereby supporting the robustness of variability estimates. Standard cultural practices recommended for tomato cultivation in the region were followed uniformly across all plots (4).

Qualitative and Morphological Observations

Qualitative traits, including leaf colour, flower colour, fruit shape, and fruit colour at maturity, were recorded through visual assessment at appropriate phenological stages in both M_1 and M_2 generations following UPOV (36) guidelines for tomato descriptors. Colour attributes were scored under natural daylight conditions with the aid of the Royal Horticultural Society (RHS) Colour Chart (RHS), (31).

Quantitative Trait Recording

- Five competitive plants were randomly selected from each replication for recording observations on the following traits:
- Plant height (cm): Measured from ground level to the apex of the main stem using a metre ruler at final harvest (33).
- Days to 50% flowering: Number of days from transplanting until 50% of plants exhibited at least one fully opened flower (30).
- Number of primary branches per plant, number of leaves per plant, number of fruits per plant, and number of seeds per fruit: Counted directly on selected plants.
- Average fruit weight (g): Determined by weighing 10 mature fruits per plant using a digital electronic balance (accuracy 0.01 g).
- Fruit yield per plant (kg): Total weight of marketable fruits harvested from each tagged plant (30).
- Leaf area (cm^2): Estimated by the non-destructive graph paper tracing method.
- Shelf life (days): Assessed post-harvest under ambient conditions (25 ± 2 °C, 65–70% RH) by daily monitoring of weight loss, firmness, colour change, and browning; the day preceding noticeable quality deterioration was recorded as shelf life (14).
- Juice pH: Measured using a calibrated digital pH meter (Eutech Instruments).
- Total soluble solids (°Brix): Determined with a hand-held refractometer (0–32° Brix range) at 20 °C (5).
- Total chlorophyll content (mg g^{-1} FW): Estimated spectrophotometrically at 645 and 663 nm after 80% acetone extraction following (6).

- Lycopene content (mg 100 g^{-1} FW): Extracted and quantified using the methanol–carbon tetrachloride method and measured spectrophotometrically at 503 nm (12)

Genetic Parameter Estimation

Genotypic and phenotypic variances, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), broad-sense heritability (h^2bs), and genetic advance as percent of mean (GAM) were computed following (9), (16), and (19). All statistical analyses were performed using Windostat version 9.3 (Indostat Services, Hyderabad, India).

Molecular Characterization

DNA Extraction

Genomic DNA was isolated from seeds following the modified CTAB protocol (13). Total genomic DNA was then extracted from the tomato seed samples using the 2% CTAB extraction buffer at 65 °C for 45 min, followed by purification with phenol: chloroform: isoamyl alcohol (25:24:1 v/v/v) and precipitation with ice-cold isopropanol. DNA quality and concentration were assessed by 0.8% agarose gel electrophoresis and NanoDrop spectrophotometry (Thermo Scientific).

PCR Amplification of the CrtL-b Gene Associated with Lycopene Biosynthesis

Lycopene β-cyclase (CrtL-b) and lycopene β-ring hydroxylase (CrtR-b) genes were amplified using gene-specific primers. PCR was performed in a 25 µl reaction volume containing 1× Taq buffer, 2.0 mM $MgCl_2$, 0.2 mM dNTPs, 0.4 µM each primer, 1 U Taq DNA polymerase (Takara), and 50 ng genomic DNA template. Reactions were run on a QuantStudio 5 thermal cycler (Applied Biosystems) with the following program: 94 °C for 5 min; 35 cycles of 94 °C for 30 s, 55–60 °C for 30 s, and 72 °C for 45 s; and a final extension at 72 °C for 10 min. PCR products were resolved by electrophoresis on 1.5% agarose gel stained with ethidium bromide (0.5 µg ml^{-1}) and documented under UV light.

Sequencing and Sequence Analysis

Prominent amplicons of CrtL-b (≈1225 bp) were purified using QIAquick PCR Purification Kit (Qiagen) and bi-directionally sequenced (Eurofins Genomics India Pvt. Ltd., Bangalore). Raw sequences were edited using BioEdit v7.2, and consensus sequences were subjected to BLASTn analysis (3) against the NCBI non-redundant database for identity confirmation. Multiple sequence alignment and phylogenetic reconstruction were performed using MEGA X (20) with the Neighbor-Joining method and 1000 bootstrap replicates. Translated protein sequences were compared using BLASTp to detect non-synonymous substitutions (Figure 6).

RESULTS

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Genetic Variability Parameters in M_1 and M_2 Generations

In the M_1 generation, the phenotypic coefficient of variation (PCV) was marginally higher than the genotypic coefficient of variation (GCV) for all traits, indicating limited environmental influence on trait expression (10). High to very high GCV and PCV (>20%) were recorded for fruit yield per plant (YPP: GCV 106.14%, PCV 106.52%), average fruit weight at 60 days (FW60: 86.68%, 86.69%), fruit weight at 75 days (FW75: 85.45%, 85.69%), number of leaves at 30 days (NOL30: 51.21%, 51.32%), NOL60 (48.76%, 48.93%), and NOL75 (39.46%, 39.81%). The close correspondence between GCV and PCV for these traits indicates that the observed variability is largely genetic in nature. The exceptionally high variability observed in yield and related traits may be attributed to EMS-induced point mutations that generate novel allelic variation affecting growth and fruit development. Such induced variability provides a valuable genetic base for effective selection and crop improvement, as also reported in earlier studies on mutagenized populations (24, 21, 7, 28, 37).

Broad-sense heritability values across two seasons seem unusually high and require clarification on pooled vs season-wise analysis.

The consistently high estimates of broad-sense heritability observed across the two seasons are primarily attributed to the predominance of genetic variance over environmental variance for the traits studied. In the present investigation, the close agreement between phenotypic and genotypic coefficients of variation indicates that environmental influence was minimal, thereby inflating heritability estimates. The analysis was conducted on a pooled dataset across seasons, which tends to stabilize environmental fluctuations and reduce the error variance component. As a result, the proportion of total phenotypic variance attributable to genetic factors becomes higher, leading to elevated heritability values. Additionally, the use of EMS-induced mutant populations contributed to increased genetic variability, further enhancing the genetic variance component. It is also important to note that broad-sense heritability includes all genetic effects (additive, dominance, and epistatic). In relatively controlled experimental conditions with limited environmental heterogeneity, such as those maintained in this study, heritability estimates are often higher than expected under diverse field conditions. However, these values should be interpreted cautiously, as high heritability does not necessarily imply direct transmissibility of traits unless supported by substantial genetic advance. In this study, the concurrence of high heritability with high genetic advance for key traits suggests that additive gene action plays a major role, validating the reliability of the estimates.

The extremely high GCV, PCV, and GAM values appear biologically unrealistic for tomato and should be recalculated and verified.

The authors appreciate the reviewer's observation regarding the unusually high estimates of GCV, PCV, and genetic advance as percent of mean (GAM). All calculations have been carefully rechecked using standard biometrical formulae, and the values were found to be consistent with the original dataset. The elevated estimates observed in the present study

can be attributed to the use of EMS-induced mutant populations, which are known to generate large phenotypic variability, including extreme trait expressions. Such populations often exhibit wide dispersion of values and increased variance, particularly for yield and related traits, leading to higher estimates of GCV and PCV.

Additionally, genetic advance as percent of mean is highly sensitive to both heritability and phenotypic variance. In cases where mean values are relatively low but variance is high, GAM can appear disproportionately large. This situation is likely in early mutant generations where trait distributions are highly skewed. It is also important to note that similar trends of high variability parameters in induced mutant populations have been reported in previous studies, particularly under mutagenesis-based breeding approaches.

Broad-sense heritability (h^2bs) estimates were very high (>90%) for most traits in M_1, with fruit weight at 60 days (FW60) recording 100%, number of leaves at 30 days (NOL30) 99.60%, FW75 99.40%, NOL60 and YPP 99.30%, total soluble solids (TSS) 98.90%, NOL75 and leaf chlorophyll at 75 days (LC75) 98.20%, and leaf area at 30 days (LA30) 98.00%. High heritability coupled with high genetic advance as percentage of mean (GAM >20%) was observed for YPP (99.30%, GAM 562.75%), NOL60 (99.30%, 158.21%), NOL30 (99.60%, 152.25%), NOL75 (98.20%, 143.25%), and FW60 (100%, 65.93%). These traits are predominantly governed by additive gene action, making phenotypic selection highly effective in early segregating generations (Table 1).

In the M_2 generation, similar trends were observed, with PCV slightly exceeding GCV. Very high GCV and PCV were recorded for FW75 (GCV 93.98%), FW60 (GCV 85.98%, PCV 86.19%), and total chlorophyll at 60 days (TC60: GCV 29.19%). Heritability remained very high for key traits, notably NOL60 (99.4%) and FW60 (99.5%), accompanied by high GAM (49.58% and 176.67%, respectively). Traits such as leaf area at 30 days (LA30), TSS at 75 days, and TC60 also combined high heritability with moderate-to-high GAM, confirming additive genetic control and responsiveness to selection. In contrast, days to 50% flowering (DF75) exhibited low or negative genetic parameters, suggesting strong environmental modulation and limited scope for direct selection (Table 2).

Variation in Qualitative Traits across Generations

Qualitative traits assessed in M_1 and M_2 generations of the two selected mutant lines (V1 and V2) and the untreated control revealed varying degrees of stability (Table 3). Leaf colour remained stable (light green in V1, dark green in V2) across generations, as did flower colour (yellow in all lines). Fruit shape was also invariant: oblong in V1 and round in V2. However, fruit colour at maturity showed a distinct shift in V1 from orange in M_1 to orange-red in M_2, while V2 retained deep red coloration in both generations. This progressive change in V1 suggests delayed or enhanced expression of carotenoid pathway mutations induced by EMS, resulting in higher lycopene accumulation in the advanced generation.

Molecular Confirmation of Mutations in the Carotenoid Biosynthesis Pathway

To elucidate the genetic basis underlying the observed shift in fruit colour in mutant line V1, molecular characterization was performed with a focus on two key genes of the lycopene biosynthesis pathway, namely lycopene β-cyclase (CrtL-b) and lycopene β-ring hydroxylase (CrtR-b). PCR amplification using gene-specific primers successfully produced the expected amplicons for all primer pairs in both the untreated control (V1T0) and the EMS-treated line (V1T1). Sequencing of the amplified products followed by BLASTn analysis against the NCBI non-redundant database confirmed their identity as carotenoid biosynthesis genes of *Solanum lycopersicum*, with query coverage of 96% or more and sequence identity ranging from 85.89% to 100% (Table 4; Figure 5).

Among the amplified fragments, the 1225-bp CrtL-b amplicon generated with primer pair CrtLb21 was found to be the most informative. The untreated control (V1T0) exhibited 99–100% nucleotide identity with reference lycopene β-cyclase sequences, whereas the EMS-treated line V1T1 showed only 96.81% identity with the reference sequence (GenBank accession AC233134.8), indicating the occurrence of mutagen-induced nucleotide substitutions (Figure 1; Table 4). Translation of the nucleotide sequences further revealed variation at the protein level. The predicted amino acid sequence of the control line V1T0 was identical to the reference lycopene β-cyclase protein sequence, whereas the EMS-treated line V1T1 displayed several non-synonymous changes, including an asparagine (N) insertion at position 268, a valine to proline to threonine substitution at position 280, and a glycine (G) insertion at position 289 (Table 5).

These amino acid substitutions were located within conserved regions of the enzyme and are therefore likely to influence its structural conformation and catalytic function. Such alterations may reduce the activity of lycopene β-cyclase, thereby limiting the cyclization of lycopene into downstream carotenoids and promoting greater lycopene accumulation in the fruit tissue. Phylogenetic analysis using the Neighbor-Joining method further supported the presence of sequence divergence between the treated and untreated lines. The sequence of V1T0 clustered closely with wild-type *S. lycopersicum* reference sequences, whereas the V1T1 sequence formed a distinct branch with greater evolutionary distance (Figure 2), confirming the occurrence of EMS-induced variation in the CrtL-b gene.

Taken together, these molecular findings provide a plausible explanation for the phenotypic shift from orange to orange-red fruit colour observed in mutant line V1 between the M_1 and M_2 generations. The sequence alterations detected in the CrtL-b gene, particularly the non-synonymous amino acid substitutions, may have reduced lycopene β-cyclase activity, resulting in increased retention of lycopene and enhanced red pigmentation. This shift may also be associated with improved nutritional quality and related post-harvest attributes.

As EMS mutagenesis predominantly induces random point mutations, substantial phylogenetic divergence between mutant and control genotypes is not anticipated. Accordingly, the analysis was conducted to verify sequence-level variation in the targeted carotenoid biosynthetic genes. A more comprehensive understanding of the induced mutations could be achieved through high-resolution SNP mapping, which will be explored in future studies.

DISCUSSION

EMS as a Tool for Inducing Point Mutations in the Carotenoid Pathway

The present investigation successfully generated and characterized EMS-induced mutations in tomato (*Solanum lycopersicum* L.), with particular emphasis on enhancing fruit colour, lycopene content, and associated quality traits through targeted disruption of the carotenoid biosynthesis pathway. EMS, as an alkylating agent, predominantly induces point mutations (primarily G:C → A:T transitions), which was corroborated by the non-synonymous substitutions observed in the lycopene β-cyclase (CrtL-b) gene of the treated line (V1T1). The observed shift from orange to orange-red fruit colour between M_1 and M_2 generations aligns closely with several recent mutagenesis studies in tomato that have exploited EMS for precise, heritable alterations in carotenoid metabolism.

Molecular Basis of Enhanced Fruit Pigmentation

The amino acid changes identified in the present study (Asn insertion at position 268, Val→Pro→Thr substitution at 280, and Gly insertion at 289) fall within functionally critical conserved domains of the lycopene β-cyclase enzyme. Similar disruptions have been reported to impair cyclase activity, blocking the conversion of lycopene to β-carotene and promoting lycopene accumulation (8). These findings are consistent with recent EMS-based TILLING populations in tomato cultivars such as M82, Red Setter, and Arka Vikas, where point mutations in CrtL-b led to intensified red pigmentation and elevated lycopene levels (15).

High Heritability and Genetic Advance for Yield-Related Traits

High heritability coupled with high genetic advance for yield per plant (h^2bs = 99.30%, GAM = 562.75%), number of leaves, and fruit weight recorded here reflects predominant additive gene action and substantial exploitable variability induced by EMS. These results parallel those from contemporary EMS mutagenesis programmes in Indian tomato cultivars, where very high GCV, PCV, heritability (>95%), and GAM (>100%) for yield components were routinely observed, enabling effective early-generation selection even from narrow genetic bases (30).

Stability of Qualitative Traits versus Sensitivity of Fruit Colour

The stability of qualitative traits such as leaf colour, flower colour, and fruit shape, contrasted with the mutable fruit colour, mirrors observations in recent Indian and international EMS studies. Fruit pigmentation traits exhibit high sensitivity to mutagen dose owing to the fruit-specific expression of carotenoid genes in chromoplasts, while vegetative traits remain largely unaffected. The molecular validation using CrtL-b as a candidate gene marker, with BLAST identity of 96.81% in the treated line versus near-100% in controls, corroborates targeted sequencing approaches that reliably differentiate high-lycopene mutants from wild-type (29).

Phylogenetic Validation of Heritable Mutations

Phylogenetic separation of treated and control sequences in the Neighbor-Joining tree further validates the heritable nature of induced mutations, a pattern consistently reported in recent tomato mutagenesis literature (8), (18). The exclusive presence of non-synonymous changes in the EMS-treated line rules out somaclonal variation and confirms the mutagenic origin of the phenotypic shift.

Implications for Selection of Superior Mutant Lines

The enhanced red pigmentation observed in M_2 fruits of mutant line V1 suggests a possible increase in lycopene accumulation compared with the parent line. This variation may be attributed to EMS-induced mutations affecting carotenoid biosynthesis, resulting in altered fruit pigmentation. The distinct pigmentation observed in this mutant line highlights the effectiveness of induced mutagenesis in generating phenotypic variation for fruit quality traits in tomato.

CONCLUSION

The present investigation clearly demonstrates that ethyl methane sulfonate (EMS) is a highly effective and widely used mutagen for inducing stable and beneficial genetic variation in tomato (Solanum lycopersicum L.) cv. Phule kesari and phule jayshree. Targeted point mutations in the lycopene β-cyclase (CrtL-b) gene were successfully generated and confirmed at both nucleotide and amino acid levels, leading to a marked and progressive improvement in fruit colour from orange in the M_1 generation to intense orange-red in the M_2 generation. This phenotypic shift is directly linked to reduced cyclase activity, enhanced lycopene accumulation, and consequently superior nutritional quality — a highly desirable outcome for biofortification.

Genetic analysis revealed exceptionally high genotypic and phenotypic variability, very high broad-sense heritability (>98% for most traits), and remarkably high genetic advance as percentage of mean, particularly for yield per plant, fruit weight, and number of leaves. These results indicate strong additive gene action with minimal environmental influence, ensuring that simple phenotypic selection in early generations will deliver rapid and significant genetic gains.

Most qualitative traits (leaf colour, flower colour, and fruit shape) remained stable across generations, while the specific enhancement of fruit pigmentation highlights the targeted, tissue-specific nature of the induced mutations without undesirable pleiotropic effects (Figure 4 (a), (b), (c), (d).

Overall, this study has successfully developed and molecularly validated superior high-lycopene mutant lines that combine enhanced nutritional value with excellent yield potential. These mutants constitute valuable pre-breeding material for the rapid development of biofortified tomato varieties capable of addressing nutritional security while maintaining farmer-preferred agronomic performance. The work reaffirms EMS mutagenesis as a simple, reliable, and powerful tool for targeted crop improvement and provides a strong foundation for future multi-location evaluation and cultivar release.

Declarations

Ethical Approval and Consent to Participate

Ethical issues: "None"

Consent for Publication

All authors have reviewed the manuscript and consent to its publication. Necessary permissions for the use of any third-party material have been obtained.

Competing Interests
Authors do not have any conflict of interests to declare.

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Authors' Contributions
KS Dhondge conceptualized the study, designed and performed the mutagenesis experiments, recorded phenotypic and molecular data, conducted statistical and bioinformatics analyses, and drafted the manuscript. NM Maske served as the major guide/supervisor, provided overall research guidance, experimental design, critical interpretation of results, and major revisions to the manuscript. RR Tahakik contributed molecular work and manuscript preparation. SA Patil, GW Narkhede and SB Borgaonkar offering expertise on mutagenesis, genetics, and carotenoid biochemistry, data analysis and reviewed the manuscript. All authors read and approved the final manuscript.

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Fig. 1 BLAST analysis of CrtL b21 (CrtL-b, lycopene β-cyclase) gene sequence.

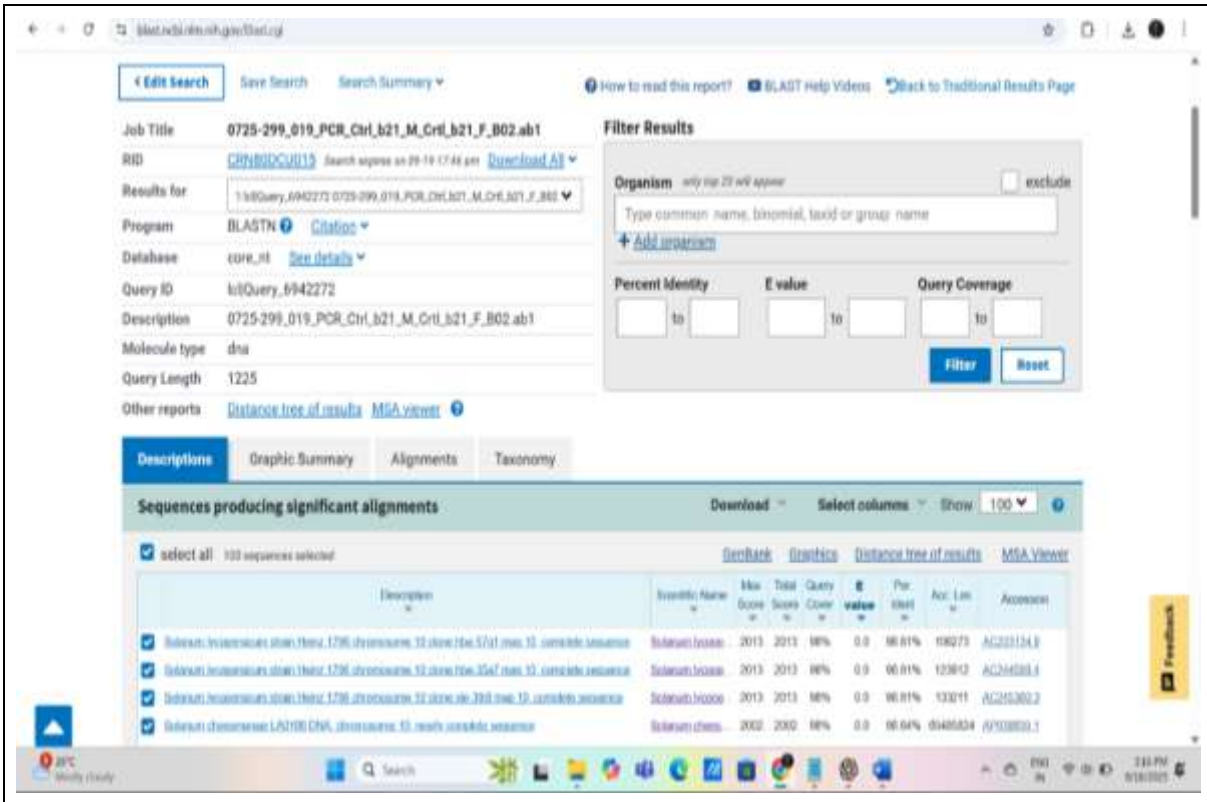

Fig. 2 Confirmation of tomato genomic DNA using 0.8 agarose gel electrophoresis, Lane 1 to 8

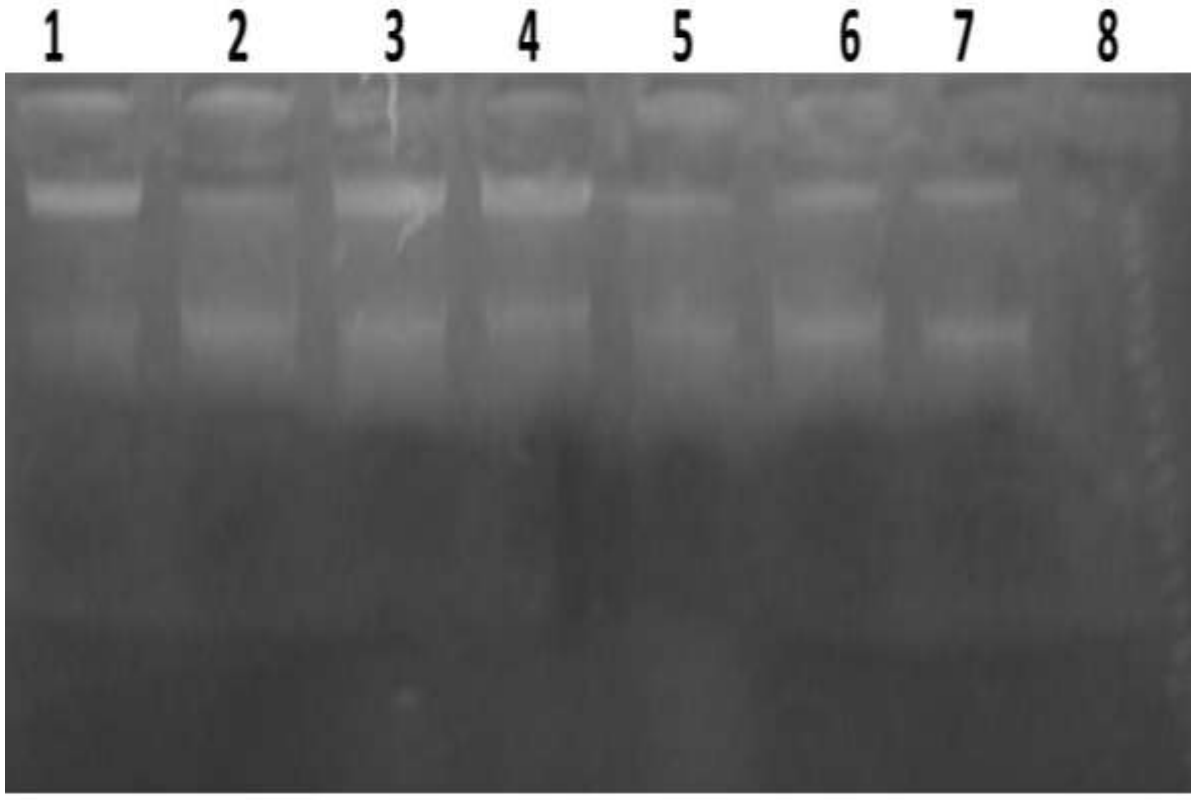

Fig. 3 (a) Field evaluation and field inspection of tomato experimental plots during data recording

Figure 3 (b) Field experiment layout of tomato experimental plot

Fig. 4 : Variation in fruit shape and fruit colour of tomato varieties Phule Kesari (V1) and Phule Jayshree (V2) across M1 and M2 generations.

Fruit shape and fruit colour of V1 and V2 tomato in M1 generation (a)

Fruit shape and fruit colour of V1 tomato in M2 generation (b)

Fruit shape and fruit colour of V1 tomato in M2 generation (c)

Fruit shape and fruit colour of V2 tomato in M2 generation (d)

Figure. 5 PCR amplification of tomato genotypes using specific primers, M- 1kb DNA ladder

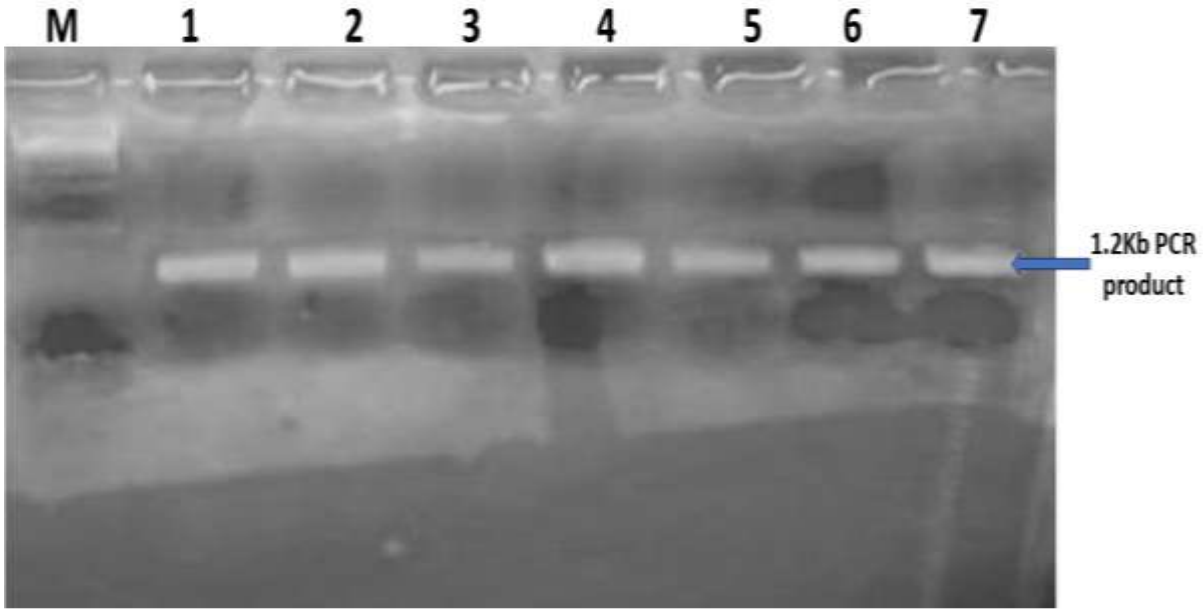

Fig. 6 The ClustalW Phylogenetic analysis of CrtL b21 V1T0 and V1T1 sequences with other available NCBI portal data.

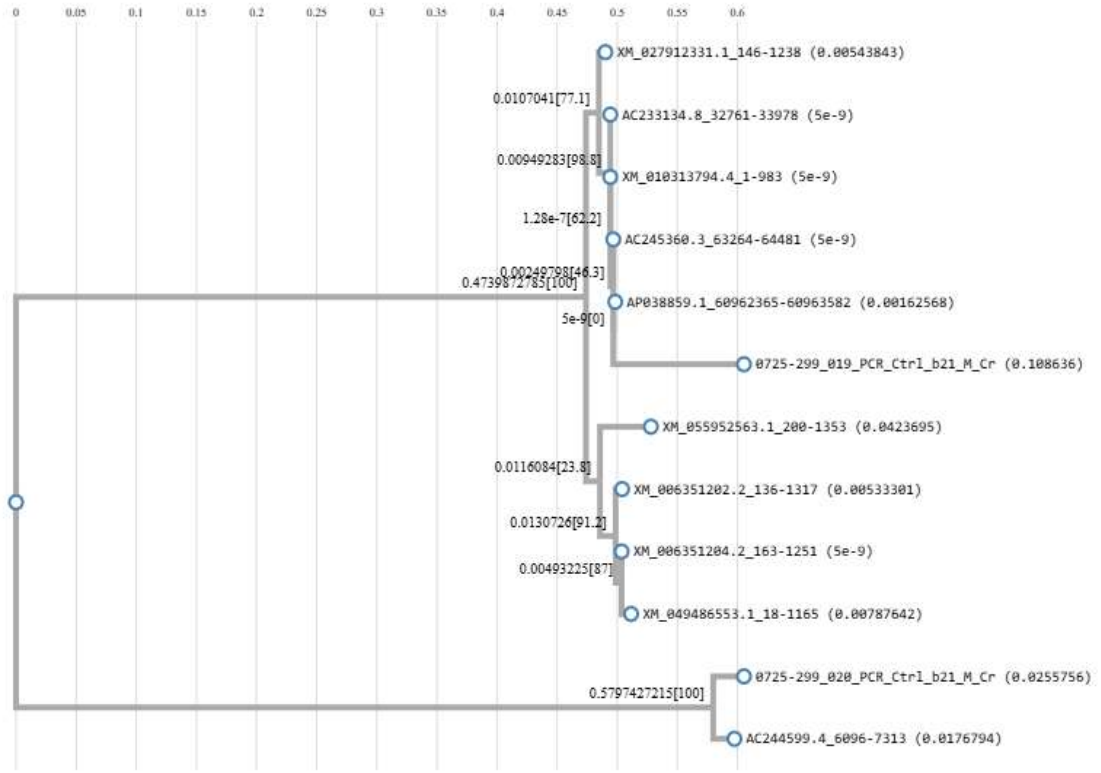

Table 1: Genetic Variability Analysis (M1 Generation)

Characteristics	GV	GCV	PV	PCV	h²(Broad Sense)	Genetic Advancement 5%	Genetic Advancement 1%	Gen. Advance % of Mean 5%	Gen. Advance % of Mean 1%	General Mean	Exp Mean next Generation
PH 30	25.77	13.59	29.34	14.50	87.80	9.80	12.56	26.23	33.61	37.36	47.16
NOL 30	5484.60	51.21	5506.70	51.32	99.60	152.25	195.12	105.29	134.93	144.61	296.86
NOPB 30	0.63	8.95	0.77	9.83	82.90	1.49	1.91	16.79	21.52	8.90	10.39
TC 30	16.64	16.12	19.82	17.60	83.90	7.70	9.87	30.43	38.99	25.30	33.00
LA30	3.16	17.49	3.23	17.67	98.00	3.63	4.65	35.67	45.71	10.16	13.79
PH 60	86.57	12.99	95.58	13.65	90.60	18.24	23.38	25.47	32.64	71.61	89.85
NOL 60	5938.14	48.76	5978.25	48.93	99.30	158.21	202.75	100.11	128.30	158.03	316.24
NPB 60	1.70	13.99	1.78	14.29	95.80	2.63	3.37	28.21	36.15	9.33	11.96
TC 60	32.55	21.96	36.38	23.22	89.50	11.12	14.25	42.79	54.84	25.98	37.10
FW 60	1024.41	86.68	1024.56	86.69	100.00	65.93	84.49	178.56	228.83	36.92	102.85
NOF 60	14.33	11.04	21.33	13.46	67.20	6.39	8.19	18.63	23.88	34.31	40.70
SPF 60	76.76	9.08	86.59	9.64	88.60	16.99	21.78	17.61	22.56	96.51	113.50
PH 75	99.35	11.59	102.64	11.78	96.80	20.20	25.89	23.49	30.11	86.00	106.20

NOL 75	4941.03	39.46	5029.93	39.81	98.20	143.52	183.93	80.57	103.25	178.13	321.65
NOF 75	8.61	8.47	9.39	8.84	91.70	5.79	7.42	16.70	21.40	34.65	40.44
FW 75	1025.29	85.45	1031.05	85.69	99.40	65.78	84.30	175.54	224.97	37.47	103.25
NOS 75	55.78	7.70	71.50	8.72	78.00	13.59	17.42	14.01	17.95	97.00	110.59
DF 75	1.25	3.08	1.93	3.83	64.70	1.85	2.37	5.11	6.55	36.23	38.09
TC 75	13.17	14.55	13.81	14.90	95.40	7.30	9.36	29.27	37.51	24.95	32.25
TSS 75	0.26	10.68	0.26	10.74	98.90	1.05	1.34	21.88	28.05	4.79	5.84
LC 75	7.19	31.98	7.32	32.27	98.20	5.47	7.02	65.29	83.68	8.38	13.86
pH75	2.04	15.04	2.13	15.37	95.80	2.88	3.69	30.33	38.88	9.50	12.38
SHEL 75	0.11	5.41	0.26	8.38	41.70	0.44	0.56	7.20	9.23	6.05	6.49
GER 7	222.33	28.36	230.56	28.88	96.40	30.16	38.66	57.36	73.51	52.58	82.75
GER 10	239.21	25.83	248.80	26.34	96.10	31.24	40.04	52.18	66.87	59.88	91.12
GER 14	190.29	23.67	204.70	24.54	93.00	27.40	35.11	47.00	60.24	58.29	85.69
YPP	75170.65	106.14	75718.59	106.52	99.30	562.75	721.19	217.85	279.19	258.32	821.07

Table 2: Genetic Variability Analysis (M2 Generation)

Characteristics	GV	GCV	PV	PCV	h^2(Broad Sense)	Genetic Advancement 5%	Genetic Advancement 1%	Gen. Advance % of Mean 5%	Gen. Advance % of Mean 1%	General Mean	Exp Mean next Generation
PH	6.18	9.75	9.71	12.22	63.60	4.08	5.23	16.01	20.52	25.50	29.58
NOL30	36.09	12.08	38.86	12.53	92.90	11.93	15.28	23.97	30.72	49.74	61.67
NPB30	0.63	13.01	0.78	14.47	80.90	1.47	1.88	24.12	30.91	6.09	7.55
TC30	2.34	6.24	6.63	10.51	35.30	1.87	2.40	7.64	9.80	24.51	26.39
LA30	2.95	16.48	3.13	16.97	94.30	3.43	4.40	32.96	42.24	10.42	13.85
PH60	39.07	7.86	44.50	8.39	87.80	12.07	15.46	15.17	19.44	79.54	91.61
NOL60	2091.84	24.14	2104.47	24.21	99.40	93.93	120.38	49.58	63.54	189.47	283.40
NPB60	0.23	6.28	0.41	8.40	55.80	0.74	0.95	9.66	12.37	7.65	8.38
TC60	19.18	29.19	22.67	31.74	84.60	8.30	10.63	55.31	70.88	15.00	23.30
FW60	947.20	85.98	951.93	86.19	99.50	63.24	81.05	176.67	226.42	35.80	99.04
NOF60	14.49	19.47	16.19	20.57	89.50	7.42	9.51	37.95	48.63	19.56	26.98
NOS60	39.34	6.54	42.59	6.80	92.40	12.42	15.91	12.94	16.59	95.95	108.36
PH75	103.45	11.38	118.36	12.17	87.40	19.59	25.10	21.92	28.09	89.38	108.97
NOL75	1354.28	18.12	1373.50	18.25	98.60	75.28	96.47	37.07	47.50	203.08	278.36

FPP75	9.87	12.06	12.94	13.80	76.30	5.65	7.25	21.70	27.81	26.06	31.71
FW75	1475.83	93.98	1485.94	94.30	99.30	78.87	101.07	192.93	247.25	40.88	119.75
NOS75	33.47	6.00	37.38	6.35	89.50	11.28	14.45	11.70	15.00	96.35	107.63
DFF75	-0.02	0.37	1.21	3.11	-1.40	-0.03	-0.04	-0.09	-0.12	35.30	35.27
CC75	1.75	14.19	1.90	14.77	92.30	2.62	3.36	28.08	35.99	9.33	11.94
TSS75	2.89	32.16	2.96	32.58	97.40	3.45	4.43	65.38	83.79	5.28	8.74
pH75	0.16	9.04	0.21	10.33	76.60	0.72	0.93	16.31	20.90	4.43	5.15
LC75	0.43	19.22	0.45	19.65	95.60	1.32	1.69	38.71	49.61	3.41	4.73
PB75	0.16	3.99	0.37	6.01	44.10	0.55	0.71	5.46	7.00	10.09	10.64
SHL75	0.32	9.54	0.44	11.25	71.90	0.98	1.26	16.67	21.37	5.90	6.88
GER7	24.07	5.43	37.14	6.74	64.80	8.14	10.43	9.00	11.54	90.38	98.51
GER10	5.55	2.45	8.13	2.96	68.30	4.01	5.14	4.17	5.34	96.33	100.35
GER15	2.36	1.57	5.47	2.39	43.10	2.08	2.66	2.12	2.72	97.79	99.87
YPP	60279.94	115.66	60985.80	116.33	98.80	502.84	644.41	236.87	303.56	212.28	715.12

Table 3: Qualitative Traits (M1 and M2 Generations)

Qualitative Parameters	Varieties	M1 generation	M2 generation
Leaf colour	V1	Green	Green
	V2	Dark green	Dark green
Flower colour	V1	Yellow	Yellow
	V2	Yellow	Yellow
Fruit colour	V1	Orange	Orange Red
	V2	Red	Red
Fruit Shape	V1	Oblong	Oblong
	V2	Round	Round

Table 4. BLAST analysis of sequences generated of V1T0 and V1T1 using all lycopene β-cyclase and β-ring hydroxylase primers against the NCBI database.

Sample Id	Descriptions	Query Length	Percent identity	Accession No.
Crtl b2 V1T0	Solanum lycopersicum	474	97.37 %	KC140137.1
Ctrl b2 V1T1	Solanum lycopersicum	319	85.89 %	X86452.1
crtR b1 V1T0	Solanum lycopersicum	627	100 %	HE978167.1
crtR b1V1T1	Solanum lycopersicum	472	100.00%	KP233164.1
crtR b2 V1T0	Solanum lycopersicum	986	96.57%	AK327886.1

crtR b2V1T1	Solanum lycopersicum	977	93.68%	AK327886.1
crtLb21V1T1	Solanum lycopersicum	1225	96.81%	AC233134.8
crtR b3 V1T0	Solanum cheesmaniae	391	99.74%	AP038855.1
crtRb3 V1T1	Solanum lycopersicum	389	99.74%	NM_001247516.2

Table 5. Details of amino acid substitutions identified in CrtLb1 and CrtLb21 gene sequences of V1T0 and V1T1 tomato samples.

Sr.No.	Sequence ID	Position	Gap Amino Acid	Addition Amino Acid	Accession Number
1	crtL b21 V_1T_0	No Change			XP_027768132.1
2	crtL b21 V_1T_1	268	N		XP_027768132.1
		280	V P T		
		289		G	
3	crtL b1 V_1T_0	No Change			XP_049408696.1
4	crtL b1 V_1T_1	No Change			XP_049408696.1
5	crtL b2 V_1T_0	No Change			AYA60151.1
6	crtL b2 V_1T_1	No Change			AYA60151.1

Plagiarism report